Accepted Manuscript

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PII: S0924-2244(18)30590-9

DOI: https://doi.org/10.1016/j.tifs.2019.02.053

Reference: TIFS 2459

To appear in: *Trends in Food Science & Technology*

Received Date: 24 August 2018

Revised Date: 20 February 2019

Accepted Date: 28 February 2019

Please cite this article as: Papoutsis, K., Mathioudakis, M.M., Hasperué, J.H., Ziogas, V., Non-chemical treatments for preventing the postharvest fungal rotting of citrus caused by *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold), *Trends in Food Science & Technology*, https://doi.org/10.1016/j.tifs.2019.02.053.

ABSTRACT

Background: Citrus is one of the most economically important horticultural crops in the world. Citrus are vulnerable to the postharvest decay caused by *Penicillium digitatum* and *P. italicum*, which are both wound pathogens. To date, several non-chemical postharvest treatments have been investigated for the control of both pathogens, trying to provide an alternative solution to the synthetic fungicides (imazalil, thiabendazole, pyrimethanil, and fludioxonil), which are mainly employed and may have harmful effects on human health and environment.

Scope and approach: The current study emphasizes the non-chemical postharvest treatments, such as irradiations, biocontrol agents, natural compounds, hot water treatment (HWT), and salts, on the prevention of decay caused by *P. digitatum* and *P. italicum*, also known as green and blue molds, respectively. The mode of action of each technique is presented and comprehensively discussed.

Key findings and conclusions: *In vivo* and *in vitro* experiments in a laboratory scale have shown that the control of green and blue molds can be accomplished by the application of non-chemical treatments. The mechanisms of action of the non-chemical techniques have not been clearly elucidated. Several studies have mentioned that the application of non-chemical treatments results in the synthesis of secondary metabolites with antifungal activities (i.e. polyphenols, phytoalexins) in fruit surface. Moreover, non-chemical treatments may exert direct effects on fungal growth, such as disruption of cell walls, inhibition of metabolic respiration, and disruption of energy production related enzymes.

Non-chemical treatments for preventing the postharvest fungal rotting of citrus caused by *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold)

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Keywords: green mold, blue mold, oranges, postharvest, sustainable treatments

1. Introduction

Citrus is one of the most important crops in the world with a global production exceeding 140 million tonnes (FAO, 2016). After harvest, citrus fruit are stored and handled in packing houses in order to maintain their postharvest life and quality, as well as to reduce the decay due to pathogen infection. *Penicillium digitatum* Sacc. (green mold) and *P. italicum* Wehmer (blue mold) are the most economically important pathogens in citrus, resulting in significant postharvest losses (up to 30 and 80%, respectively) (El-Otmani, Ait-Oubahou, & Zacarías, 2011). Both *P. digitatum* and *P. italicum* are wound pathogens which produce a large amount of airborne spores (conidia) reproduced asexually and infect the fruit through the wounds made by insects, branches, or inappropriate human handling during harvest (Kellerman, Joubert, Erasmus, & Fourie, 2016).

The control of blue and green molds is currently accomplished by the pre- and postharvest application of chemical fungicides, such as imazalil, thiabendazole, pyrimethanil, and fludioxonil (Berk, 2016). However, the extensive pre- and postharvest usage of chemical fungicides on citrus has caused the development of resistant fungi strains resulting in a breakdown of fungicide efficiency (Hao, Li, Hu, Yang, & Rizwan-ul-Haq, 2011; Sánchez-Torres & Tuset, 2011). Therefore, methods for monitoring fungicide baseline sensitivity for all postharvest fungi including *Penicillia* should be conducted. Although these methods are very expensive and time consuming, they are necessary to prolong over time the technical life of fungicides (Vitale, Panebianco, & Polizzi, 2016; Piccirillo et al., 2018). Additionally, currently consumers are concerned about the consumption of fruit sprayed with fungicides, since their active compounds and co-formulants have been associated with several health issues and environmental pollution (Nicolopoulou-Stamati, Maipas, Kotampasi, Stamatis, & Hens, 2016). Furthermore, citrus export companies are adopting more strict policies regarding

77 pesticide residues, which is in accordance with the public concern of safer agricultural
78 commodities (Tripathi & Dubey, 2004; Palou, Smilanick, & Droby, 2008; Talibi, Boubaker,
79 Boudyach, & Ait Ben Aoumar, 2014). The use of chemical substances with potential
80 carcinogenic or endocrine-disrupting effects may possess an unknown threat to human health.
81 Furthermore, the determination of "safe" levels only for a single chemical phytosanitary
82 compound underestimates the real health hazard provoked via the chronic exposure and
83 consumption of several chemical compounds (Nicolopoulou-Stamati et al., 2016). Thus, there
84 is a need to establish alternative postharvest decay control methods, as standalone procedures
85 or coupled with other means with low toxicity and environmental awareness (Palou et al.,
86 2008). Over the last two decades, several studies have been conducted investigating the effect
87 of non-chemical treatments, such as irradiations, natural compounds, biocontrol agents, hot
88 water treatment (HWT), and salts, on the control of blue and green molds (Pavoncello, Lurie,
89 Droby, & Porat, 2001; Hao et al., 2011; Talibi et al., 2014; Jeong, Chu, Lee, Cho, & Park,
90 2016).

91 The current study focuses on the control of the postharvest pathogens *P. digitatum* and
92 *P. italicum* by the use of non-chemical techniques (biocontrol agents, irradiations, salts, plant
93 extracts, essential oils, and HWT). The mechanisms of action implicated in the control of the
94 postharvest pathogens are also presented and discussed (Figure 1).

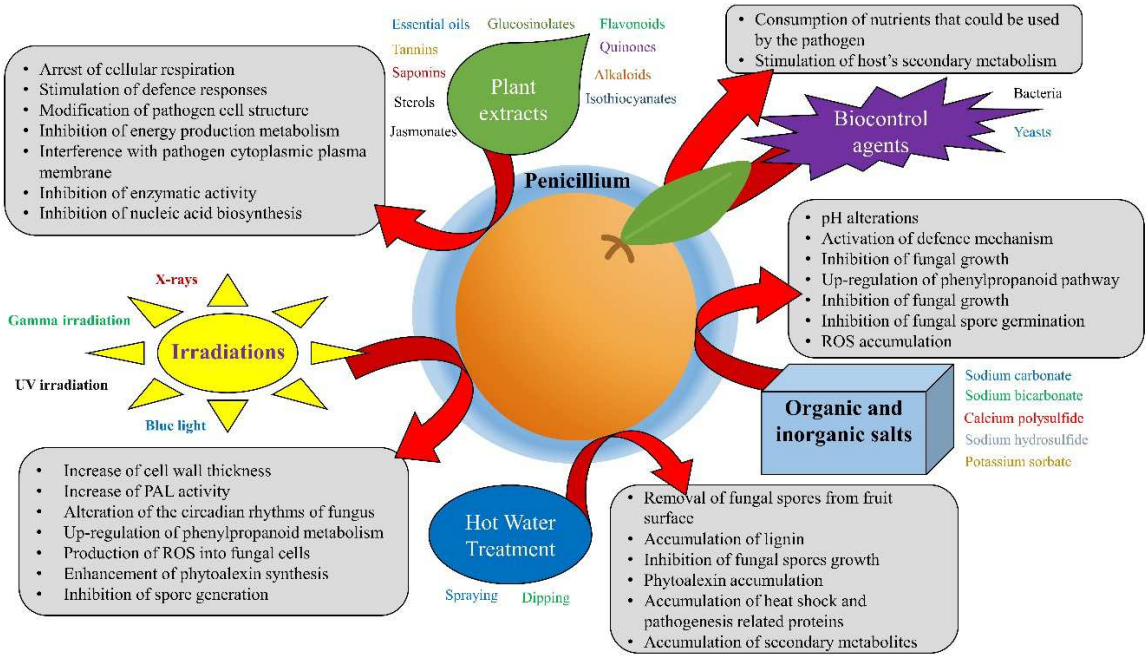

Figure 1. Schematic illustration of mechanisms of action for different non-chemical treatments against *Penicillium* in citrus.

2. Pathogenicity of *Penicillium digitatum* and *Penicillium italicum*

P. digitatum and *P. italicum*, causing the green and blue molds respectively, are fungi classified in the order Eurotiales and the Trichocomaceae family (Palou et al., 2008; Talibi et al., 2014). Both citrus *Penicillium* molds are wound pathogens, which can only infect fruit through rind wounds during field harvesting, packing handling or commercialization, or injuries made on fruit surfaces 2-3 days before harvest by physiological conditions (e.g. cold, wind) and insects (Kellerman et al., 2016). Fungal spores, massively produced by rotten fruit (fallen on the ground of orchard, packing house, storage room), are airborne disseminated and they can easily cause contamination of the surrounding fruit at any stage, before or after harvest. The severity of the forthcoming disease development generally depends on the amount of pathogen spores established on the rind wounds, the fruit maturity (mature fruits are highly susceptible) and the optimum temperature conditions (20-25 ^{o}C) for pathogen infection (Kellerman et al., 2016). Although for both pathogens the optimum temperature for

112 germination and growth is 25 ^{o}C, green mold is more favored at ambient temperatures, as
113 conidia germination and hyphal growth development is faster, whereas blue mold gets more
114 important at lower cold-storage conditions. The infection site appears as a soft, watery, and
115 decolorized spot due to the production of pathogenic hydrolytic enzymes (e.g.
116 polygalacturonase, glucosidase), which cause maceration of tissues and facilitates their
117 colonization by fungi, eventually leading to fruit decay. Within the wide utilization of
118 volatiles as antifungal agents (detailed in section 3.2), there is an exception. Certain specific
119 monoterpene volatiles released from the citrus peel (as limonene, myrcene, pipene), have been
120 reported to be important to the germination and growth of *P. digatatum* and *P. italicum*, with
121 the former being more sensitive to the stimulatory effect of citrus volatiles than the latter
122 (Droby et al., 2008). Specifically, the germinated spores of *P. digitatum* and *P. italicum* were
123 75.1% and 37.5%, respectively, when the fungi were exposed to citrus peel volatiles
124 compared to the controls (6.8% and 14.7%, respectively). However, the same volatiles had an
125 inhibitory effect in the germination of *P. expansum* and *Botrytis cinerea* (Droby et al., 2008).
126 Taking into consideration that volatiles derived from citrus peel caused an increase in spores
127 germination of *P. digitatum* and *P. italicum*, whereas volatiles derived from non-pathogen
128 hosts had no effect in combination with the specific stimulatory effect of citrus peel volatiles
129 solely on citrus pathogens (*P. digitatum*, *P. italicum*) and the inhibitory effect on non-citrus
130 pathogens (*P. expansum*, *B. cinerea*), Droby et al. (2008) suggested that these citrus peel
131 monoterpene volatiles serve as signaling molecules in host recognition by *P. digitatum* and *P.*
132 *italicum*. Despite the different mold color of the sporulating area between *P. digitatum* and *P.*
133 *italicum*, the former is surrounded by a thick non-spolurating mycelium limited by a decaying
134 peel, while the latter is surrounded by a sparse non-sporulating mycelium limited by a soft,
135 watery peel (Palou et al., 2008; Talibi et al., 2014). During disease development, fruit surface
136 is fully covered with spores followed by shrink initiation, which leads to a sunken mummified

form in case of green mold, whereas in the case of blue mold, the mummified form becomes a sticky mass. The whole process is relative humidity dependent. It is noteworthy that blue mold can spread in healthy fruit in storage boxes more frequently and by direct attack (only by contact) in contrast to green mold, in which case the contamination of adjacent fruit is rare. Moreover, *Penicillium* spp. are considered as great producers of extrolites, including mycotoxins and other secondary metabolites, which can be toxic and harmful to humans and animals (Barkai-Golan, 2008; Perrone & Susca, 2017). *P. digitatum* and *P. italicum* have not been included among the mycotoxigenic producers but other extrolites have been reported such as the tryptoquialanins by the former, and deoxybrevianamide E, italinic acid, formylxanthocillin X and PI-3 by the latter (Frisvad & Samson, 2004).

3. Control of *P. digitatum* and *P. italicum* by the use of natural compounds

3.1. Use of plant extracts

Mother Nature has always been a valuable source for humans toward the search for useful compounds in order to overcome problems linked with food preservation. During the last decades, several reports have indicated that the use of plant extracts is a potential alternative method for the efficient management of citrus postharvest diseases (Ameziane et al., 2007). Extensive work has been done towards the effectiveness of plant extracts to participate into the development of innovative antifungal compounds that could be used in order to control citrus postharvest diseases. Plant extracts originating mainly from medicinal and aromatic plants have been implemented as preventative methods toward the development of postharvest decays of citrus fruit and showed encouraging results during *in vitro* and *in vivo* studies (Askarne et al., 2013; Li Destri Nicosia et al., 2016). The application of plant extracts as autonomous potential fungicides or in combination with other control measures is rather promising due to their well-documented antifungal activity, low phytotoxicity, systemic mode of action, decomposability, and low environmental toxicity (Tripathi & Dubey, 2004;

Askarne et al., 2012). Those latter attributes make plant extracts valuable assets in the arsenal of the sustainable agriculture because it mostly exploits natural cycles with reduced environmental impact (Li Destri Nicosia et al., 2016).

Several reports suggest the ability of aqueous or organic solvent extracts from different plants to control citrus decay caused by *P. italicum* and *P. digitatum* due to their content in secondary metabolites such as flavonoids, quinones, tannins, terpenes, alkaloids, saponins, sterols, phenylpropanoids, acetaldehyde, benzaldehyde, benzyl alcohol, ethanol, methyl salicylate, ethyl benzoate, ethyl formate, hexanal, (E)-2-hexanal, lipoxygenases, jasmonates, allicin, glucosinolates, isothiocyanates, verbascocide, and isoverbascocide (Tripathi & Dubey, 2004; Palou et al., 2008; Talibi et al., 2014; Li Destri Nicosia et al., 2016). *In vivo* and *in vitro* studies on grapefruits showed that the application of low doses of jasmonates (jasmonic acid and methyl jasmonate) is an effective method to control citrus decay caused by *P. digitatum* (Droby, Wisniewski, Macarisin, & Wilson, 2009). Kanan & Al-Najar (2009) reported how methanolic extracts from cinnamon bark (*Cinnamomum cassia* L.), sticky fleabane leaves (*Inula viscosa* L.), and harmal seeds (*Peganum harmala* L.) were able to inhibit the growth of fungal isolates of *P. italicum* upon infected lemons and oranges. The high fungitoxic activity of *I. viscosa* crude and methanolic extracts against *P. italicum* was related to the high content of phenolics, flavonoids, and anthraquinones, while the antifungal activity of *P. harmala* extract was attributed to the high concentration in phenolics and alkaloids (Kanan & Al-Najar, 2009).

Many studies have attributed the antifungal activity of plant extracts to the presence of polyphenols. Sanzani, Schena, & Ippolito (2014) reported that phenolic compounds such as quercetin, scopoletin, and scoparone exerted antifungal activity toward *P. digitatum* on navel oranges. Pomegranate peel extract has drawn the attention of many research groups for the quest of potential, sustainable, and alternatives to chemical fungicides due to its high

antioxidant activity and antimicrobial capacity correlated with the high concentration of phenolics (Li Destri Nicosia et al., 2016; Tayel, Moussa, Salem, Mazrou, & El-Tras, 2016; Pangallo et al., 2017). Phenolic extracts from pomegranate peels inhibited the conidia germination of *P. digitatum* and *P. italicum* and delayed the overall decay of the artificially inoculated grapefruits and lemons (Li Destri Nicosia et al., 2016; Tayel et al., 2016; Pangallo et al., 2017). The absence of any phytotoxic syndrome upon the tested citrus proves the capability of pomegranate peel extract to be used as an effective eco-friendly and food safe control agent against postharvest citrus rots (Li Destri Nicosia et al., 2016).

Even though there is extensive literature upon the beneficial effect of plant extracts toward the control of fungal infestation during postharvest storage, little is known about the mode of actions that they exert. Most of the studies that have been conducted so far have tried to elucidate the mechanism of action of polyphenols against green and blue molds. Polyphenols contained in the plant extracts may stimulate the synthesis of secondary metabolites with antifungal activities in the fruit tissue, as well as it may have detrimental effects on the morphology and growth of fungi (Yang et al., 2016). In the work of Pangallo et al. (2017), phenolic extracts from pomegranate peels applied to citrus fruit enhanced the defense mechanisms into flavedo (the outer colored part of the peel). This justifies the fact that pomegranate peel extract has the ability to stimulate resistance cascades to fruit tissues, and those responses are linked with the opposed inhibition of pathogen development and infection upon the fruit (Pangallo et al., 2017). Pomegranate peels extracts resulted in the over-accumulation of reactive oxygen species (ROS), and the increased expression of five genes: chitinase (*CHI*), chalcone synthase (*CHS*), mitogen-activated protein kinase (*MAPK*), mitogen-activated protein kinase kinase (*MAPKK*), and phenylalanine ammonia-lyase (*PAL*), all related with the activation of plant defense responses (Pangallo et al., 2017). It was suggested that pomegranate peel extracts were able to exert their mode of action via the

induction of resistance mechanisms in fruit tissue via the priming effect (Pangallo et al., 2017). Priming is the cellular state in which the harmful effects of abiotic stress factors in plants are hindered by pre-exposure to a stimulus, thus resulting in greater survival levels (Tanou et al., 2009). Priming techniques (use of natural or artificial compounds) have been related with the more efficient activation of defence syndromes, thus enhancing the ability to tolerate forthcoming stress factors (Tanou et al., 2009; Ziogas et al., 2015). MAPK cascades have been linked with plant defence responses, resulting in the synthesis of pathogen related (PR) protein, the production of ROS, and even cell death. Also, under adverse conditions, plant tissues increase the production of phytoalexins via the modulation of PAL and CHS, enzymes involved in the biosynthesis of phenolics, whose participation in resistance mechanisms of citrus to biocontrol factors and adverse conditions has already been proposed (Hershkovitz et al., 2012). Therefore, the proposed induced resistance mechanism due to the application of pomegranate peel extract may constitute a promising curative measure due to the rapid activation of defence cascades that may prevent or even minimize the potential of the fungi to colonize the host tissue (Li Destri Nicosia et al., 2016; Pangallo et al., 2017). Yang et al. (2016) reported that in the presence of poplar bud plant extracts which were rich in flavonoids without B-ring substituents (pinocembrin, chrysin and galangin), the hyphae of *P. italicum* became shrivelled and wrinkled while the cell membrane became severely disrupted. The authors suggested that flavonoid plant extracts exerted their mode of action via the disruption of cell membrane permeability, the arrest of metabolic respiration, and the disruption of energy production related enzymes of fungus (Yang et al., 2016).

Taking into account the fact that plant extracts consist of a mixture of different compounds, it is easier to speculate that the antifungal activity should be the outcome of a plethora of possible modes of actions. In general, those modes of action are linked with: i) the stimulation of defence responses (Pangallo et al., 2017), ii) the inhibition of nucleic acid

biosynthesis via the inhibition of DNA gyrase (Wu, Zang, He, Pan, & Xu, 2013), iii) the ability to interfere with the cytoplasmic plasma membrane of the pathogen, inducing alternation in fluidity and outflow of intercellular substances (Cushnie & Lamb, 2005), iv) the modification of pathogen cell structure (Xu, Zhou, & Wu, 2011), v) the arrest of cellular respiration and initiation of oxidative stress syndromes (Yang et al., 2016), vi) the inhibition of energy production metabolism (Yang et al., 2016), and vii) the inactivation of enzymes, causing disruption of the functionality of the genetic material (Telezhenetskaya & D'yakonov, 1991).

The justification of the ability of a plant extract to act as efficient antifungal agent is the first step toward the development of a natural commercial viable eco-friendly product (Li Destri Nicosia et al., 2016). However, there are several aspects of the botanical merits that need attention and certain obstacles must be surpassed, including the following: i) the applied product must be effective even after short term treatment application, ii) the quality parameters of the fruit should not be negatively affected, iii) the utilized effective dose must be as low as possible, iv) the efficacy of the applied natural product should not be affected by the environmental conditions or fruit physiology, vii) the applied natural extract should have low residual activity and not be toxic for human health (i.e. alkaloids), and viii) it should be considered the specificity of action versus targeted pathogen, and not have wide fungal activity against multiple phytopathogens (Tripathi & Dubey, 2004; Talibi et al., 2014; Li Destri Nicosia et al., 2016) (Table 1).

Table 1. Mode of actions and required characteristics of an ideal plant extract against *P. digitatum* and *P. italicum*.

		References
Mode of Actions	Stimulate defence responses	(Pangallo et al., 2017)
	Inhibit nucleic acid biosynthesis	(Wu et al., 2013)
	Interfere with pathogen cytoplasmic plasma membrane permeability	(Cushnie & Lamb, 2005)
	Modify pathogen cell structure	(Xu et al., 2011)

	Inhibit cellular respiration	(Yang et al., 2016)
	Initiate oxidative stress syndromes	(Yang et al., 2016)
	Inhibit energy production metabolism	(Yang et al., 2016)
	Inactivate of essential enzymes	(Telezhenetskaya & D'yakonov, 1991)
	React with cell membrane proteins	(Telezhenetskaya & D'yakonov, 1991)
	Disrupt function of genetic material	(Telezhenetskaya & D'yakonov, 1991)
Required Characteristics	Effective after short term treatment application	(Talibi et al., 2014)
	No negative effect upon fruit quality attributes	(Talibi et al., 2014)
	Low effective dose	(Tripathi & Dubey, 2004)
	Efficiency not affected by environmental conditions or fruit physiology	(Li Destri Nicosia et al., 2016)
	Low residual activity – not toxic to humans	(Li Destri Nicosia et al., 2016)
	Specificity of action versus targeted pathogens	(Li Destri Nicosia et al., 2016)

3.2. Use of essential oils

Essential oils (EOs) are natural volatiles, oil soluble substances produced into various plant organs and are known for their antibacterial, antifungal, antiviral, insecticidal, antioxidant, and medicinal properties (Yahyazadeh, Zare, Omidbaigi, Faghih-Nasiri, & Abbasi, 2009; Talibi et al., 2014). Recent studies have proven that EOs have two unique attributes: i) they are natural products, safe for consumers, and ecosystem and ii) there is minor risk of resistance development by postharvest pathogens due to various volatile substances which exist within the essential oil (EO) mixture, each exerting a different antifungal mode of action (Tripathi & Dubey, 2004; Yahyazadeh et al., 2009). The volatile nature of EOs and their high biodegradability make them efficacious and advantageous postharvest antifungal agents for citrus industry with low levels of traceable residues (Talibi et al., 2014). The successful usage of EOs as efficacious postharvest fungicides has been reported to many citrus species like satsuma mandarin (Shao et al., 2015), orange (Cháfer, Sánchez-González, González-Martínez, & Chiralt, 2012), sour orange (Trabelsi, Hamdane, Said, & Abdrrabba, 2016), and lemon (Pérez-Alfonso et al., 2012) (Table 2).

In the work of Boubaker et al. (2016), the antifungal activities of EOs from four *Thymus* species were investigated against *P. digitatum* and *P. italicum*. *In vitro* experiments showed that fungal spore germination varied significantly between the different EOs used from

Thymus species, but all of them were able to effectively control both *P. digitatum* and *P. italicum* (Boubaker et al., 2016). Also, several EOs derived from lemon grass, eucalyptus, clove, and neem were tested for their ability to inhibit fungal growth of green and blue molds upon the surface of kinnow mandarins (Jhalegar, Sharma, & Singh, 2015). This study revealed that all EOs were able to inhibit mycelial growth and conidia germination of both pathogens upon the surface of citrus fruit, with the concentrations used being able to negatively affect various developmental stages of the pathogens. Among the different EOs, lemon grass EO was the most competent to exert the most beneficial effect toward the control of green and blue molds (Jhalegar et al., 2015).

Tabti et al. (2014) evaluated the effect of an EO derived from *Thymus capitatus* on oranges artificially infected with *P. italicum*. In the applied EO mixture consisting of 38 compounds, carvacrol was the most predominant and drastic, further supporting its previously demonstrated antifungal capacity against *Penicillium spp.* (Marković et al., 2011). Tripathi and Dubey (2004) screened and evaluated the effect of EOs from *Mentha arvensis*, *Ocimum canum*, and *Zingiber officinale* as botanical fungitoxicants against the postharvest rooting of citrus fruit. All the observed EOs were able to control blue mold infections on oranges and limes, extending their commercial shelf life and supporting the usage of EOs as potential economical fungitoxicants (Tripathi & Dubey, 2004). Trabelsi et al. (2016) highlighted the importance of EOs extracted from different plant parts (flower, peel, and leaves) of sour orange (*Citrus aurantium* L.) against *P. italicum* and *P. digitatum*. The dominant compound in flower EO was linalool, in the peel was limonene, and in the leaves was linalyl acetate. The effect of each EO was tested *in vivo* upon sour oranges and the results demonstrated that EOs derived from the leaves and flowers were able to reduce the growth of *P. italicum* and *P. digitatum*, while the peel EO extract was ineffective against both pathogens (Trabelsi et al., 2016). The inability of limonene to arrest the mycelia growth of *P. italicum* and *P. digitatum*

was also reported by Droby et al. (2008). Monoterpene volatiles, especially limonene, strongly stimulate germ elongation and exert the role of a messenger molecule in host recognition procedures by both pathogens (Droby et al., 2008; Trabelsi et al., 2016). Interestingly, other reports have stated that the EOs of oregano, cinnamon, and clove were ineffective against the fungal growth of *P. digitatum* and *P. italicum* in oranges (Plaza, Torres, Usall, Lamarca, & Viñas, 2004a; Yahyazadeh et al., 2009). Compared with other studies, the EOs used for the control of *P. digitatum* and *P. italicum* upon citrus should not be applied at high concentration, since there is always the risk of phytotoxicity and increased application cost (Yahyazadeh et al., 2009).

Several studies propose an alternative approach to EO application via the usage of wax or other compounds such as chitosan that could minimize the volatility and increase effectiveness and duration of the EOs upon the surface of the citrus fruit (Cháfer et al., 2012; Tao, Fan, Jia, & Zhang, 2014; Shao et al., 2015; Grande-Tovar, Chaves-Lopez, Serio, Rossi, & Paparella, 2018). Cháfer et al. (2012) supported the combination of EOs with film components, since the positive antifungal effect that is observed in *in vitro* studies cannot be found *in vivo*. This could be attributed to the increased volatility of EOs and their possible interactions with the vegetative tissues. Tao et al. (2014) demonstrated that the utilization of wax with the EO octanal can exhibit performance similar to a fungicide against *P. digitatum* when applied on Satsuma mandarin. The application of octanal embedded into postharvest wax decreased the fungal growth of *P. digitatum* upon Satsuma mandarins and improved citrus fruit quality characteristics (vitamin C content, coloration index, total soluble solid content, and pH) (Tao et al., 2014). Also, the combination of chitosan with several EOs was presented by Cháfer et al. (2012) and Shao et al. (2015). In the work of Cháfer et al. (2012), chitosan was mixed with different EOs originated from bergamot, thyme, and tea tree. The application of these EOs mixed with chitosan upon the surface of oranges before and after the

329 inoculation of the fruit with *P. italicum* resulted in a significant delay of fungal decay and
330 preserved fruit quality parameters throughout the cold storage period. Shao et al. (2015)
331 reported that the application of chitosan combined with clove oil resulted in citrus fruit
332 resistance against *P. digitatum*. Specifically, the combination of 1% chitosan with 0.5 mL L^{-1}
333 clove oil reduced the fungal lesions and enhanced the activity of PAL and CHI. The
334 application of EOs via embedded coatings was further tested in a study conducted by
335 Yahyazadeh et al. (2009), in which EO vapors in polyethylene bags with nano-clay particles
336 were able to control *Penicillium* decay on citrus fruit. However, it should be considered that
337 the type of the polyethylene film could alter the sensory characteristics of the citrus fruit
338 (Yahyazadeh et al., 2009). In order to improve the efficiency of the application of EO within
339 wax, other factors, such as formulation solubility, gas permeability, compound compatibility
340 between EOs and waxes, should be taken into account (Kouassi, Bajji, & Jijakli, 2012).

341 The mode of action by which EOs exert their antifungal effect against *P. digitatum* and
342 *P. italicum* when treated upon the surface of citrus fruit is a matter of debate. Tao et al. (2014)
343 proposed that the mechanism of action of EOs against fungi is based upon the disruption of
344 the cell membrane integrity and membrane permeability. It has been suggested that the
345 lipophilicity of EOs facilitates their infiltration from the aqueous phase into the membrane
346 structure of fungi. This infiltration results in several intercellular negative consequences, like
347 membrane enlargement, increase of membrane fluidity and permeability, disturbance of
348 membrane-embedded proteins, respiration arrest, disruption of ion transport processes and an
349 overall leakage of ions or other intercellular contents (Tao et al., 2014; Shao et al., 2015).

350 Scientific data suggest that a more complex interplay of volatile compounds acting as
351 stimulants or inhibitors between plant and pathogen interactions may exist since limonene, α-
352 pinene, β-pinene, and myrcene were suggested for being signaling molecules responsible for
353 host recognition by *P. italicum* and *P. digitatum* (Droby et al., 2008), and limonene synthase

down regulation in *Citrus sinensis* improved fruit resistance to *P. digitatum* (Rodriguez et al., 2011).

After evaluating the overall antifungal activity of EOs against *P. digitatum* and *P. italicum*, it can be suggested that EOs are promising candidates towards the search for alternative solutions to chemical fungicide. EOs are considered as Generally Recognized As Safe (GRAS), eco-friendly compounds that could replace chemical fungicides. Their commercial application may facilitate in the overall management of postharvest decay caused by green and blue molds and minimize health hazard that exists due to the high usage of chemical compounds by citrus industry. However, an open scale commercial usage of EOs as antifungal agents must be under tight control due to potential problems that may occur related with phytotoxicity, unpleasant odors, or limited ability to implement technologies that will allow the fumigation of vast amounts of produce or the use of aquatic media (Palou et al., 2008).

Table 2. Summary of studies on the effect of essential oils on *P. digitatum* and *P. italicum*.

Fruit	Target pathogen	Essential oil	References
Orange, lime	*P. italicum*	*Mentha arvensis, Ocimum canum, zingiber officinale*	(Tripathi & Dubey, 2004)
Orange	*P. digitatum, P. italicum*	*Cinnamomum zeyla, nicum*	(Kouassi et al., 2012)
Satsuma mandarin	*P. digitatum*	Octanal	(Tao et al., 2014)
Orange	*P. digitatum, P. italicum*	*Citrus aurantium*	(Trabelsi et al., 2016)
Satsuma mandarin	*P. digitatum*	Clove oil	(Shao et al., 2015)
Orange cv. Thompson, orange cv. Valencia	*P. digitatum, P. italicum*	*Thymus vulgaris, Eugenia caryophyllata* Thunb	(Yahyazadeh et al., 2009)
Orange cv. Salustiana, Orange cv. Valencia	*P. digitatum, P. italicum*	*Thymus vulgaris, Cinnamonum zeylanicum* Breyn	(Plaza et al., 2004a)
Orange cv. Tomango	*P. digitatum*	*Mentha spicata, Lippia scaberrima*	(du Plooy, Regnier, & Combrinck, 2009)

Orange cv. Navel Powell	*P. italicum*	Bergamot, Thyme,Tea tree	(Cháfer et al., 2012)
Lemon cv. Fino	*P. digitatum, P. italicum*	Carvacrol, Thymol	(Pérez-Alfonso et al., 2012)
Orange	*P. italicum*	*Thymus capitatus*	(Tabti et al., 2014)

4. Control of *P. digitatum* and *P. italicum* by the use of irradiations

Several studies have pointed out that the application of non-ionizing (UV-C, UV-B, blue light) and ionizing irradiations (gamma, and X-rays) have the potential of reducing the amount of fungal diseases in citrus (Table 3) (Rojas-Argudo et al., 2012; Gündüz & Pazir, 2013; Liao, Alferez, & Burns, 2013; Yamaga, Kuniga, Aoki, Kato, & Kobayashi, 2016; Jeong et al., 2016). The efficiency of decay reduction in the fruit is mainly influenced by the irradiation type, as well as its penetration ability (Jeong et al., 2016). The following sections attempt to discuss the possible action mechanisms of each irradiation type, plus potentials for commercial use.

4.1. Non-ionizing irradiation

4.1.1. Ultraviolet irradiation (UV)

UV is a non-ionizing irradiation divided into UV-C (100–280 nm), UV-B (280–315 nm), and UV-A (315–400 nm). UV irradiation is perceived by vegetative tissues through photoreceptors and regulates several metabolic pathways. During the UV treatment, citrus are placed underneath the UV lamp for a specified amount of time, varying from a few seconds to a few hours (Arcas, Botía, Ortuño, & Del Río, 2000; D'Hallewin, Schirra, Pala, & Ben-Yehoshua, 2000; Ruiz et al., 2017). The UV intensity that reaches the surface of the fruit is influenced by the distance between the UV lamp and the fruit, as well as the time that the lamp is on (Gündüz & Pazir, 2013). Both UV-C and UV-B irradiations have been extensively studied on the prevention of citrus decays caused mainly by *P. digitatum* and *P. italicum*. The

efficiency of the UV treatment against green and blue molds might be affected by several parameters, such as UV irradiation type and intensity, harvesting period, maturity stage of fruit, depth of the infection in the peel, and storage temperature during the first 24h following the UV treatment (Droby et al., 1993; D'Hallewin et al., 2000; Gündüz & Pazir, 2013; Yamaga et al., 2016). Although UV-C irradiation prevents the decay of citrus commodities caused by the green and blue molds, high intensities may cause damage on the flavedo of citrus (Rodov, Ben-Yehoshua, Kim, Shapiro, & Ittah, 1992). For instance, Gündüz & Pazir (2013) reported that UV-C irradiation of 7.92 kJ m^{-2} significantly reduced the decay caused by both green and blue molds of oranges, however, higher UV-C intensities negatively affected the quality of the fruit. On the other hand, UV-B irradiation seems to have less harmful effects on the surface of the citrus fruit compared to UV-C (Kaewsuksaeng, Urano, Aiamla-or, Shigyo, & Yamauchi, 2011). UV-B irradiation has been proven to reduce the incidence of both green and blue molds in lemons and satsuma mandarin, respectively (Ruiz et al., 2016; Yamaga et al., 2016).

Several mechanisms are involved in the resistance of citrus against green and blue molds following UV treatment and they could be divided into direct and indirect. As a direct mechanism, the effect of UV irradiation could be considered when it is absorbed by the surface of fungus. In *in vitro* experiments, Yamaga et al. (2016) found that UV-B irradiation higher than 30 kJ m^{-2} inactivated the conidia of *P. italicum* and *P. digitatum*. On the other hand, UV irradiation induces metabolic and anatomical changes in citrus flavedo which are involved in fruit resistance against pathogens (indirect mechanism) (Rodov et al., 1992; Droby et al., 1993; Kovács & Keresztes, 2002; Ruiz et al., 2016). Ruiz et al. (2016) found that UV-B irradiation of lemons resulted in the thickening of flavedo cell walls creating a barrier for the pathogen. Additionally, after UV treatment secondary metabolites with antifungal activities, such as polyphenols and phytoalexins, are accumulated in the flavedo of the fruit

(D'Hallewin et al., 2000; Ruiz et al., 2016; Ruiz et al., 2017). Given that UV-C irradiation implicated in phytochemical reactions, the incubation temperature after treatment is crucial for the initial 24 h following the treatment. For instance, Droby et al. (1993) reported that UV-treated grapefruits kept for the initial 24 h after treatment at 6 °C were more susceptible to *P. digitatum* than those stored at higher temperatures. Although UV irradiation is an effective treatment for the control of citrus postharvest fungi, there are several issues that must be addressed before this method is used by food industry. For example, the distrust of consumer towards irradiated fruit should be overcome. Future studies investigating the effect of UV treatment on citrus decay should be conducted on a commercial and/or large scale. Also, the optimum dosages which are able to control the postharvest decay of citrus without affecting the quality of the product, should be reported.

4.1.2. Blue light

Blue light (400–500 nm) is a part of the visible spectrum and regulates several metabolic processes into vegetative tissues (Lafuente & Alférez, 2015; El-Esawi et al., 2017). Several studies have mentioned that blue light could be applied for the control of both *P. digitatum* and *P. italicum* (Liao et al., 2013; Yamaga, Takahashi, Ishii, Kato, & Kobayashi, 2015; Ballester & Lafuente, 2017). The mechanisms of blue light effects on the control of citrus decay have yet to be elucidated. However, it could be hypothesized that the resistance induced after blue light treatment might be due to a direct effect of light on fungal growth or an indirect effect of light on fruit elicit resistance, or both (Liao et al., 2013; Lafuente & Alférez, 2015; Ballester & Lafuente, 2017). *In vitro* experiments have shown that blue light affects fungal morphology and sporulation, while the efficacy of the treatment against *P. digitatum* and *P. italicum* increases with the duration of the application and with the light quantum flux (Lafuente & Alférez, 2015). These effects could be due to the implication of

blue light in the circadian rhythms and the production of ROS into fungal cells (Tisch & Schmoll, 2010; El-Esawi et al., 2017). The inhibitory effect of blue light on green and blue molds requires a direct exposure of the infected fruit surface to the light (Liao et al., 2013). Apart from the changes that the blue light induces into the fungal cells, it also regulates metabolic pathways into the plant tissues, which might be implicated in the resistance against fungi. For instance, blue light induces on treated citrus the expression of phospholipase A_2 (PLA_2) gene, which is a key element in the lipid signalling pathway and is involved in plant immunity responses (Alferez, Liao, & Burns, 2012). Moreover, high quantum flux of blue light induces the phenylpropanoid metabolism in citrus flavedo and this results in the increase of the phytoalexin scoparone, which has been linked to antifungal activities (Ballester & Lafuente, 2017).

To summarize, blue light application has the potential to reduce the decay caused by green and blue molds during the postharvest storage of citrus. Although the exact mechanism of blue light has not yet been fully understood, it could be hypothesized that blue light has a direct effect on fungal physiology and also induces the production of secondary metabolites into citrus flavedo, which are involved in fruit resistance against fungi.

4.2. Ionizing irradiation

4.2.1. Gamma-irradiation

Gamma-irradiation has been proven as a sustainable method that could be applied for the extension of postharvest life of fruit and vegetables (Guerreiro et al., 2016). Low dosage gamma-irradiation treatment may retard fruit ripening by inhibiting ethylene production and respiration rate, as well as by regulating the activity of enzymes being involved in the scavenging of free radicals (Wang, Gao, Tao, Wu, & Zhibo, 2017). To date, gamma irradiators use either cobalt-60 or cesium-137 as radioactive sources, with cobalt-60 being

dominated. Gamma-irradiation is a promising treatment for reducing postharvest decay of citrus products due to its detrimental effects on fungal physiology, and to its penetration ability (Cia, Pascholati, Benato, Camili, & Santos, 2007; Schweiggert, Carle, & Schieber, 2007). The efficiency of this method is linked to the radiosensitivity of each pathogen (Jeong, Shin, Chu, & Park, 2015). However, before this method is commercially applied, consumers' mistrust regarding irradiated foods should be overcome (Cia et al., 2007).

The effect of gamma-irradiation on the growth of green mold was recently investigated and it was found that fungal growth was inhibited in a dose-dependent manner (Jeong et al., 2016). Although a dosage of 1 kGy can effectively inhibit the growth of *P. digitatum*, it practically cannot be applied, since it causes severe damage on the surface of citrus (Jeong et al., 2016). However, these negative effects can be eliminated by operating gamma-irradiation at lower doses, in combination with other treatments. In this regard, Jeong et al. (2016) reported that a combination of 10 ppm sodium dichloro-striazinetrione (NaDCC) with 0.4 kGy gamma-irradiation significantly reduced the incidence of *P. digitatum* in mandarins. The mechanism by which gamma-irradiation inhibits fungal growth is associated with its ability to disrupt the fungal cell membrane, leading to a loss of intracellular contents (Jeong et al., 2016). Further research is, however, required regarding the elucidation of gamma-irradiation mechanisms against citrus fungi. Although the direct effect of gamma-irradiation on fungal growth has been proven, it is not clear if gamma-irradiation induces the synthesis of plant secondary metabolites with antifungal activities. Considering the negative effects of gamma-irradiation on citrus quality, future studies should focus on the elimination of the damaging effects by combining low gamma-irradiations with other environmentally friendly techniques.

4.2.2. X-rays

X-ray is an electromagnetic irradiation with frequencies of 10^{16} to 10^{19} Hz (Moosekian, Jeong, Marks, & Ryser, 2012). Previous studies on different food commodities have shown that X-ray irradiation is a novel decontamination technology, which could replace the conventional sanitizers, since it has antimicrobial activities against various pathogenic bacteria (Moosekian et al., 2012). However, the main X-ray shortcoming is related to consumer acceptance of irradiated products. The primary target of the energy (photons) being generated by the X-ray sources is water. After photon reaction with water, free hydroxyl and hydrogen radicals may be generated, which may stimulate physiological functions in the living organisms (Droge, 2002).

A few studies have been conducted investigating the effect of X-ray irradiation on the control of green and blue molds in citrus (Palou et al., 2007; Rojas-Argudo et al., 2012). Palou et al. (2007) investigated the effect of X-ray irradiations (510 and 875 Gy) in combination with sodium carbonate (3% w/v, at 20 °C for 150 s) in controlling green and blue molds during storage at different conditions. After fungal inoculation, the fruit were treated with 3% (w/v) sodium carbonate and X-ray irradiation was applied after 36 h fruit incubation at 20 °C. The combined treatment of X-ray irradiation and sodium carbonate could not be a substitute for conventional chemical fungicides, since the reduction of disease incidence on fruit either incubated at 20 °C for 7 days or cold-stored at 5 °C for 21 days was not sufficient for satisfactory disease control under hypothetical commercial conditions. The reduced efficiency of the treatment was attributed to the lower temperature of sodium carbonate solution and the high susceptibility of the fruit to decay, as well as to the rinse of the treated fruit with tap water after sodium carbonate application. Another reason for the reduced efficiency could be the application of the treatment after fungal inoculation. As it was previously mentioned, irradiations may induce the synthesis of secondary metabolites with antifungal activities. The accumulation of these compounds is favoured under specific

514 conditions and requires a certain period of time. Rojas-Argudo et al. (2012) showed that X-
515 ray irradiation of 510 Gy induced the synthesis of the phytoalexins scoparone and scopeletin
516 after 14-day storage at 20 °C, while the accumulation of these compounds was retarded when
517 the fruit were stored at 5 °C for 60 days. In general, X-ray irradiation by itself seems not to be
518 efficient for the control of blue and green molds. However, its efficiency can be increased
519 when it is combined with carbonic acid salts, such as sodium carbonate. Future studies should
520 be focused on the effect of X-ray irradiation combined with other environmentally and human
521 friendly techniques.

522

523 **Table 3.** Studies on the effect of different types of irradiations on blue and green molds.

Fungi species	Citrus species	Type of irradiation	Highlights	References
Non-ionizing irradiation				
P. digitatum & *P. italicum*	Orange	UV-C	• Low UV-C irradiation (7.92 kJ m^{-2}) effectively inactivates spores on the surface of fruit. • Inoculation method significantly affect the efficiency of UV-C treatment.	(Gündüz & Pazir, 2013)
P. digitatum	Grapefruit	UV-C	• Low dosages of UV-C irradiation induced resistance against *P. digitatum*. • UV irradiation affected the activity of PAL and POD.	(Droby et al., 1993)
P. digitatum	Bitter orange	UV-C	• UV-C irradiation reduced the growth of *P. digitatum* on previously irradiated fruit. • The changes were attributed to the changes in flavonoid levels due to UV-C irradiation.	(Arcas et al., 2000)
P. digitatum	Lemon	UV-B	• UV-B radiation resulted in the increase of phenolic compounds in the flavedo of the treated lemons. • UV-B irradiation resulted in an increase in cell wall thickness.	(Ruiz et al., 2016)
P. italicum	Mandarin	UV-B	• UV-B irradiation had inhibitory effects against *P. italicum* spore germination and hyphae growth. • UV-B irradiation did not affect fruit quality with respect to soluble solid content, titratable acidity, and peel color.	(Yamaga et al., 2016)
P. digitatum	Lemon	UV-B	• Short-time UV-B irradiation enhanced the antifungal activity of lemon peel extracts. • Extracts inhibited conidial germination and increased TBARS, ROS and membrane permeability.	(Ruiz et al., 2017)
P. digitatum	Orange	LBL	• LBL light quantum fluxes of 210 and 630 µmol m^{-2} s^{-1} resulted in the increase of scoparone in the flavedo. • Ethylene and phenylpropanoids are not critical factors in LBL-elicited response.	(Ballester & Lafuente, 2017)

P. italicum	Mandarin	LBL	• Low-intensity LBL irradiation reduced blue mold symptom development in satsuma mandarin. • LBL supressed fungal sporulation.	(Yamaga et al., 2015)
P. digitatum & *P. italicum*	*In vitro*	LBL	• The combination of high quantum flux followed by a continuous lower quantum flux may reduce both sporulation and mycelial viability.	(Lafuente & Alférez, 2015)
P. digitatum & *P. italicum*	Tangerine, orange	LBL	• LBL effectively suppressed the mycelial growth and postharvest symptom development caused by *P. digitatum* and *P. italicum* in both tangerines and oranges. • One hour exposure to LBL per day was enough to significantly reduce *P. digitatum* sporulation.	(Liao et al., 2013)
P. digitatum	Tangerine	LBL	• LBL with a peak emission at 456 nm reduced *P. digitatum* infection in harvested tangerines. • LBL treatment induced PLA_2 gene expression and reduced the infection rate.	(Alferez et al., 2012)
Ionizing irradiation				
P. digitatum	Mandarin	Gamma-irradiation	• Green mold was inhibited in a dose-dependent manner. • Gamma-irradiation of 1.0 kGy showed a complete inhibition of spore germination, germ tube elongation, and mycelial growth of *P. digitatum*. • Gamma-irradiation resulted in the loss of plasma membrane integrity, causing the release of intracellular contents such as soluble proteins. • High gamma-irradiation doses caused severe fruit damage.	(Jeong et al., 2016)
P. digitatum & *P. italicum*	Mandarin	X-ray irradiation	• X-ray irradiations of 510 and 875 Gy reduced the sporulation of both fungi on mandarins being previously treated with SC. • X-ray irradiation treatment followed by either 14 days at 20 °C or 60 days at 5 °C had no significant impact on fruit quality.	(Palou et al., 2007)
P. digitatum	Mandarins	X-ray irradiation	• X-ray irradiation treatment induces scopoletin. • Storage conditions significantly affected the synthesis and retention of scoparone and scopoletin. • The combination of 3% SC with 510 Gy proved to delay the development of *P. digitatum*.	(Rojas-Argudo et al., 2012)

UV: Ultraviolet
LBL: LED blue light
PAL: Phenylalanine ammonia-lyase
POD: Peroxidase
TBARS: Thiobarbituric acid-reactive species
ROS: Reactive oxygen species
PLA_2: Phospholipase A_2
SC: Sodium carbonate

5. Control of *P. digitatum* and *P. italicum* by the use of hot water treatment (HWT)

Hot water treatment (HWT) has been extensively applied on different fruit and vegetables to reduce the decay caused by different pathogens and prolong their storage life (Ban et al., 2015; Sui, Wisniewski, Droby, Norelli, & Liu, 2016). HWT is a physical stress,

which induces several physicochemical changes into the fruit. In citrus, the HWT can be applied during postharvest by either dipping the fruit in warm water or spraying them with warm water while they are moving on the conveyer line. The application time of the heat treatment depends on water temperature. In citrus, different water temperatures have been examined; ranging from 40 to 65 ˚C (Porat et al., 2000; Pavoncello et al., 2001; Palou, Usall, Munoz, Smilanick, & Vinas, 2002; Kyriacou, 2011; García, Olmo, & García, 2016) (Table 4). García et al. (2016) investigated the effect of different postharvest heat treatments at both laboratory and industrial scale on the decay amount and quality of different citrus varieties. Specifically, different varieties of mandarins and oranges were inoculated with *P. digitatum* and *P. italicum* and then were dipped into hot water of different temperatures for different intervals. The optimum HWT conditions were different for each variety, with the HWT of 53 and 45 ˚C for 3 min being the most efficient for the reduction of fruit decay. These treatments delayed the skin colour evolution and reduced the firmness during a 5-day and 7-day storage at 5 and 20 ˚C, respectively. However, at the same time, other quality parameters such as soluble solid content, juice content, pH, titratable acidity, and sensory quality were not affected by the heat treatment. Similarly, Nafussi et al. (2001) showed that a hot water dip (52-53 ˚C) for 2 min prevented the decay caused by *P. digitatum* in lemons during postharvest storage. Higher water temperatures can be used when fruit are sprayed with hot water. Pavoncello et al. (2001) showed that grapefruits brushed and sprayed with hot water (62 ˚C) for 20 sec developed resistance against green mold decays. Interestingly, the authors mentioned that the HWT was more efficient when fruit were inoculated with the fungus 1 or 3 days after the treatment, while the HWT was less effective when fruit were inoculated on the same day or 7 days later. The authors showed that the HWT resulted in the accumulation of CHI and *β*-1,3-glucanases, proteins which might be implicated in the resistance of grapefruits against *P. digitatum* decay. Both dipping and spraying seem to give similar results in the

562 reduction of green and blue mold decays. However, the application of hot water by spraying
563 might be more suitable to be installed in the conveyer line of packing houses (Ben-Yehoshua,
564 2003). Several mechanisms might be implicated in the citrus resistance against green and blue
565 molds during HWT. The primary mechanism of HWT is to disinfect the commodity from
566 fungal spores found on the surfaces of citrus (Pavoncello et al., 2001). Moreover, HWT
567 interrupts for 24-48 h the growth of fungal spores which have been retained after the
568 treatment on citrus surfaces. Meanwhile, the applied heat induces the accumulation of
569 secondary metabolites such as phytoalexins (scoparone and scopoletin), heat shock and
570 pathogenesis related proteins implicated in fruit resistance against fungus (Perotti et al., 2015;
571 Sui et al., 2016). Nafussi et al. (2001) reported that the accumulation of scoparone and
572 scopoletin initiates 24 h after HWT and reaches a sufficient level for fungal inhibition within
573 48 h. At the same time, the accumulation of lignin in the parts of fruit that have been infected
574 by fungus is enhanced by the HWT and works as a barrier against pathogen's further
575 invasion, protecting the fruit from an extended decay (Nafussi et al., 2001). These results are
576 in agreement with Yun et al. (2013), who mentioned that lignin and ROS play an important
577 role in citrus resistance to *P. italicum* after HWT. Future studies should be planned in order to
578 elucidate the exact mechanism of HWT involved in citrus resistance against both green and
579 blue molds. -Omics technologies, such as genomics, proteomics, and metabolomics will
580 facilitate in better understanding the molecular and biochemical processes occurring after
581 HWT in fruit and pathogens (Sui et al., 2016). Optimization experiments should be conducted
582 in order to determine the optimum water temperatures and treatment duration period for the
583 control of citrus pathogens. Also, the combination of HWT with other non-chemical
584 techniques such as UV irradiation, plant extracts, biocontrol agents, and salts should be
585 examined.
586

587 **Table 4.** Hot water treatment conditions applied on citrus.

Product	Pathogen	Hot water treatment conditions	References
Grapefruits	*P. digitatum*	Spraying and brushing for 20 s with hot water at 62 °C	(Pavoncello et al., 2001)
Lemons	*P. digitatum*	Dip in water at 52-53 °C for 2 min	(Nafussi et al., 2001)
Mandarins, Oranges	*P. digitatum*, *P. italicum*	Dip in water at 53 °C and 45 °C (appropriate temperature depends on the variety) for 3 min	(García et al., 2016)
Citrus grandis L.×C. paradisi Macf.	*Penicillium* molds	Dip in water at 52 °C for 2 min or hot drench brushing at 52, 56 or 60°C for 10 s	(Rodov et al., 2000)
Citrus reticulata Blanco × *Citrus sinensis* (L.) Osbeck	*P. digitatum*	Dip in water at 56° for 3 min	(Kyriacou, 2011)
Tangerines, oranges, and grapefruit	*P. digitatum*	Spraying and brushing for 20 s with hot water at 56, 59, and 62°C	(Porat et al., 2000)

588

589 **6. Control of *P. digitatum* and *P. italicum* by the use of salts**

590 Several organic and inorganic salts with low-toxicity (i.e. sodium bicarbonate, sodium
591 carbonate, potassium sorbate, ammonium bicarbonate, calcium polysulfide, sodium
592 ethylparaben, and sodium hydrosulfide) have been tested for citrus decay control caused by *P.*
593 *italicum* or *P. digitatum* with some of them being characterized as GRAS compounds by the
594 Food and Drug Administration (FDA) and European Union (EU) regulations (Youssef,
595 Ligorio, Nigro, & Ippolito, 2012a; Moscoso-Ramírez, Montesinos-Herrero, & Palou, 2013;
596 Youssef, Sanzani, Ligorio, Ippolito, & Terry, 2014). The potential antifungal activity of these
597 factors could be enhanced if combined with other treatments such as heat, low doses of
598 fungicides, and wax coating (Smilanick, Mansour, Gabler, & Sorenson, 2008; Youssef et al.,
599 2012a).

600 Sorbic acid salts have been used as food additives for years and are well known for their
601 ability to exert antifungal activity against molds and yeasts, mainly within the pH range of 3-

602 6.5. Interestingly, sorbic acid salts not only exert compatibility characteristics with fungicides
603 like imazalil, thiabendazole, pyrimethanil and fludioxonil, but also improve their antifungal
604 ability against *P. digitatum* (Smilanick et al., 2008). Youssef, Ligorio, Sanzani, Nigro, &
605 Ippolito (2012b) investigated the effectiveness of sodium bicarbonate, sodium carbonate,
606 sodium silicate, potassium bicarbonate, potassium carbonate, potassium sorbate, calcium
607 chloride, and calcium chelate to control postharvest decays upon clementines and oranges at
608 different time points of application (prior to harvest, after harvest, and both in pre- and
609 postharvest). The results indicated that application time is a crucial factor that should be taken
610 into account, since salts applied to the field prior to harvest have more available time to
611 interact with the pathogen upon the fruit, thus altering its inoculum density, the environmental
612 conditions into the wound niche and may induce tissue resistance (Youssef et al., 2012b).
613 Additionally, in the work of Youssef et al. (2014), the application of sodium carbonate or
614 sodium bicarbonate resulted in the significant antifungal postharvest control of *P. digitatum* in
615 oranges. Although recent studies suggest that sodium carbonate could efficiently manage *P.*
616 *digitatum* and *P. italicum* decays in lemons, oranges, and mandarins, it is well-known that this
617 salt cannot provide overall protection to the fruit from re-infection (Palou et al., 2002; Plaza,
618 Usall, Torres, Abadias, Smilanick, & Viñas, 2004b). An *in vivo* study on citrus demonstrated
619 that the food additive sodium benzoate, commonly used as preservative, was the most
620 effective salt to perform as an antifugal agent against *P. italicum* and *P. digitatum*
621 (Montesinos-Herrero, Moscoso-Ramírez, & Palou, 2016).

622 Apart from GRAS compounds, other salts could also be used as antifungal agents
623 against *Penicillium* spp. Fu et al. (2014) suggested sodium hydrosulfide (hydrogen sulfide
624 donor) as an innovative compound to be used against the postharvest pathogens of *Aspergillus*
625 *niger* and *P. italicum* when inoculated to citrus. Fumigation of mandarins or oranges with
626 hydrogen sulfide reduced the growth of *P. italicum* on the surface of the tested fruit. This

result provides a clue for the production of novel alternative formulations that could minimize postharvest fruit decay via the fumigation with hydrogen sulfide (Fu et al., 2014).

The ability of salts to perform as alternative antifungal agents has also been tested in combination with other factors such as heat, chemical fungicides or coating. The performance of potassium sorbate against *P. digitatum* was increased in heated aqueous solutions. The combination of heat and potassium sorbate salts under long immersion times resulted in better control of the disease (Smilanick et al., 2008). The combination of curing (storage at 33 °C for 65 h) and sodium carbonate resulted in the increase of the antifungal activity of carbonic salt and provided protection from re-infection (Plaza et al., 2004b). In addition, Montesinos-Herrero et al. (2016) showed that dip treatment of citrus into heated solution of sodium benzoate resulted in a significant reduction of *P. italicum* and *P. digitatum* incidence upon 'Valencia', 'LaneLate' oranges, lemons and 'Ortanique' mandarins under postharvest storage conditions, but not on 'Clemenules' mandarins.

The ability of certain salts to be combined with wax towards their antifungal activity against *Penicillium* molds upon citrus was evaluated by Youssef et al. (2012a). Potassium sorbate embedded into wax proved the most potential antifungal agent against citrus postharvest decay, but the film-forming properties of the wax were impaired, resulting in fruit weight loss (Youssef et al., 2012a; Parra, Ripoll, & Orihuel-Iranzo, 2014). Only ammonium bicarbonate was able to exert adequate antifungal properties without interfering with the ability of the wax to retard weight loss (Youssef et al., 2012a). An alternative approach towards the efficient usage of sodium bicarbonate salts was proposed by Fallanaj, Sanzani, Zavanella, & Ippolito (2013). The authors indicated that sodium bicarbonate salt coupled with electrolysis caused by conductive diamond electrodes resulted in a synergistic effect and inhibition of *Penicillium* spore germination, with no observed deleterious effect upon fruit appearance.

Even though extensive research has been conducted using different salts for the control of postharvest *Penicillium* decays upon citrus, the mode of actions of those compounds are not fully determined. It was established that osmotic stress mediated by the concentration of salts during field applications may participate into the decrease of fungal populations (Ippolito, Schena, Pentimone, & Nigro, 2005). It is also well known that fungi grow better in acidic to neutral conditions, than in alkaline ones. As regards sodium carbonate and bicarbonate, it was widely accepted that the main mode of action was exerted via the buffering capacity of the carbonate ions and the development of an alkaline environment. Under these conditions, fungi spend more energy for acid production than upon hyphal extension, thus their growth is inhibited (Talibi et al., 2014). In general, the pH of the media is a key factor but it is not the only one for a successful postharvest decay control management, since it affects the germination of conidia and influences the virulence of pathogens via their colonization upon the host tissue (Smilanick, Mansour, Margosan, Gabler, & Goodwine, 2005).

Youssef et al. (2014) proposed that sodium carbonate and bicarbonate exert their mode of action against green mold via the activation of defense mechanism and the up-regulation of phenylpropanoid pathway. The defense responses were correlated with the increase of enzymatic activity of β-1,3-glucanase, peroxidase (POD), and PAL. Also, there was an observed up-regulation of the expression level of PAL with parallel increased levels of sucrose, scoparone, and phytoalexins (Youssef et al., 2014). Additionally, Venditti, Molinu, Dore, Agabbio, and D'Hallewin (2005) reported that sodium carbonate treatment caused alternation to the cell structural components, induced biosynthesis of scoparone, and elevated pH levels in citrus albedo (the inner white part of the peel). This proposed interaction of the rid with the treatment determines the efficacy of the applied salt, and minimum efficiency

against *P. digitatum* was observed when the salt film coating was delivered upon unwounded flavedo tissues (Venditti et al., 2005).

It is speculated that the direct effect of electrolyzed sodium carbonate against *P. digitatum* and *P. italicum* is an outcome of combined modes of action. In detail, electrolyzed sodium carbonate induces oxidative stress in *P. digitatum* conidia via the over accumulation of ROS, resulting in the collapse of the mitochondrial membrane potential and the disruption of intercellular ATP production (Fallanaj et al., 2016). Furthermore, the up-regulation of defense related genes coding for CHI, POD, PAL, as well as the prevention of tissue colonization by the pathogen, supports the ability of electrolyzed sodium carbonate to participate in the induction of host resistance (Fallanaj et al., 2016).

The antifungal activity of sodium hydrosulfide was attributed to the liberated hydrogen sulfide gas, which exerts its antifungal activity by affecting multiple aspect of fungal growth, like inhibition of spore germination, germ tube elongation, mycelial growth, abnormal contraction of mycelial cytoplasm, and ROS-related mechanisms that inhibit growth of postharvest pathogens (Fu et al., 2014).

In general, it has been well established that the inhibitory ability of many salts towards fungal pathogens is directly correlated with the presence of the residues of the tested compound within the wound infection sites occupied by the pathogen and upon the interactions of the salt with the compounds of the rid (Smilanick et al., 2005). These interactions between the rid and the applied salt differ among the citrus species and cultivar due to different albedo and flavedo characteristics (peel, skin structure, and cuticle layer) and variability of compounds with antifungal activity within the citrus rid (Montesinos-Herrero, del Río, Pastor, Brunetti, & Palou, 2009). The rid properties of citrus species determine the natural susceptibility to postharvest decay and define the efficiency of the applied salt upon

the rid (Youssef et al., 2012a; Moscoso-Ramírez et al., 2013; Montesinos-Herrero et al., 2016).

There is an increased demand for more chemical-free fruit products by consumers and fruit distributors in the EU and worldwide (Montesinos-Herrero et al., 2016). More combinations of salts with wax or low doses of chemical fungicides should be performed in order to minimize fungal decays to the minimum and reach market requirements. Further studies should be performed in order to establish the chemical properties of wax layers and investigate the effective implementation of salts into the film-coated layers. To attend this need, the implementation of salts in combination with other eco-friendly antifungal agents could lead to an alternative disease control, with no tolerance to fungicide residues.

7. Control of *P. digitatum* and *P. italicum* by the use of biocontrol agents

Biological control or biocontrol is the managing of a disease by applying biological agents to a host fruit, which prevents the development of the disease caused by a pathogen (O'Brien, 2017). Various strains of yeasts and bacteria have been used as biocontrol agents against both *P. digitatum* and *P. italicum*. To date, only a few biocontrol agents (Pantovital and Biosave) are commercially available for the control of *Penicillium* in citrus (Spadaro & Droby, 2016). The efficacy of biocontrol agents in controlling the decay caused by blue and green molds is affected by several parameters such as the type of biocontrol agent (i.e. fungi, yeasts, or bacteria), the strain used for treatment, the pH of the media where the pathogen and the biocontrol agent are grown, and the time that the biocontrol agent is applied (prior or post pathogen infection) (Droby et al., 2002; Luo, Zeng, & Ming, 2012; Panebianco, Vitale, Polizzi, Scala, & Cirvilleri, 2015; Zhang, Mahunu, Castoria, Yang, & Apaliya, 2018). Although several mechanisms of action have been proposed, the mode of action by which the biocontrol agent controls the decay caused by pathogens has not been clearly understood. The competition for nutrients and space is more frequently reported as the prevalent mode of action for

both bacteria and yeasts (Droby et al., 2002; Meziane et al., 2006; Abraham, Laing, & Bower, 2010; Luo et al., 2012; Panebianco et al., 2015). Other mechanisms that may be implicated in the control of *P. digitatum* and *P. italicum* are the production of toxins and enzymes by the biocontrol agent, which might result in deformation of fungal mycelium and inhibition of fungal spore germination, as well as in the stimulation of the secondary metabolism in the infected fruit (Luo et al., 2012; Panebianco et al., 2015). In case of yeasts, direct mycoparasitism has also been reported (Droby et al., 2002). In general, it can be hypothesized that, at the same time, more than one mechanism of action may take place, resulting in the control of the pathogens. Abraham et al. (2010) screened the effect of 60 yeast and 92 *Bacillus* isolates against *P. digitatum* and found that only 10 yeast and 10 *Bacillus* isolates were efficient in the control of *P. digitatum* on oranges (navel and Valencia) and lemons, while yeast isolates were more effective than the *Bacillus* ones. However, the combination of different types of biocontrol agents (bacteria with yeasts or different strains of bacteria) may result in a significantly higher green and blue mold inhibition compared to the individually applied cultures or strains. For instance, the combination of two strains of the bacterium *Serratia plymuthica* (IC1270 and IC14) was more efficient in the control of both green and blue molds on orange than the individual bacterium strains (Meziane et al., 2006). Panebianco et al. (2015) showed that the application of a mixture of *Pseudomonas* and *Trichoderma* strains resulted in higher inhibition of *P. digitatum* on oranges and lemons than the application of the individual biocontrol agents. The application time of the biocontrol agent is a crucial parameter affecting the performance in the suppression of green and blue molds. In general, biocontrol agents should be applied prior to pathogen infection. This is might be due to the fact that the biocontrol agents consume the nutrients that could be used by the pathogen, as well as for the stimulation of host's secondary metabolism which may lead to the synthesis of metabolites with antifungal activities (Luo et al., 2012; Panebianco et al., 2015). It has been shown the activities of enzymes such as POD, polyphenoloxidase (PPO), PAL, CHI, and β-1,3-glucanase, as well as the content of flavonoids are increased in the citrus peels after the application of the biocontrol agent

Pichia membranefaciens (yeast), leading to lower *P. italicum* and *P. digitatum* growth in citrus (Luo et al., 2012). PAL is involved in phenolic compounds synthesis, which have the ability of altering fungal cell permeability, leading to macromolecule leakage (Papoutsis et al., 2018). On the other hand, both POD and PPO enzymes are responsible for the oxidation of phenolic compounds to quinones, which are also compounds with antifungal activities (Kanan & Al-Najar, 2009). Therefore, future studies should be conducted with the aim of elucidating the implication of both phenols and quinones in the control of green and blue molds. Both CHI and *β*-1,3-glucanase are pathogenesis-related proteins synthesized by the plants as a response to pathogen infection and are implicated in the hydrolysis of chitin and *β*-1,3-glucans contained in the cell walls of fungi (Luo et al., 2012).

The efficiency of biocontrol agents against green and blue molds can be enhanced by their combination with different treatments such as hot water, plant extracts, or sodium bicarbonate (Hao et al., 2011; Hong et al., 2014; Sui et al., 2016). *Bacillus amyloliquefaciens* strain HF-01 found on the surface of citrus species is a species of bacterium with antifungal activity against both *P. digitatum* and *P. italicum* (Hao et al., 2011). Hao et al. (2011) found that the combination of the biocontrol agent *B. amyloliquefaciens* strain HF-01 with tea saponins was as effective as the imazalil (fungicide) and more effective than the application of individual treatments for the control of blue and green molds in 'Wuzishatangju' mandarins. The mode of action of the combination of the biocontrol agent with the saponins has not been clearly elucidated. Saponins have the potential of inhibiting the mycelial growth and spore germination of both green and blue molds. However, at the same time, saponins as natural surfactants may facilitate better retention of the antagonist on fruit surface by increasing the wettability of the treated surface and spreading the antagonist more evenly over the fruit (Hao et al., 2011). Hong et al. (2014) showed that the efficiency of the biocontrol agent *B. amyloliquefaciens* strain HF-01 against *P. digitatum* and *P. italicum* can also be enhanced when bacterium is combined with hot water treatment at 45 °C for 2 min or/and 2% sodium bicarbonate. A recent review study conducted by Sui et al. (2016) highlighted the synergistic effect between yeasts

and heat treatment on postharvest disease control. However, to date, no studies have been conducted in citrus investigating the effect of the combination of heat treatment with yeast as a biocontrol agent on the control of blue and green molds.

Both *P. digitatum* and *P. italicum* can be controlled by the application of biocontrol agents, which could be considered as an alternative to the conventional fungicide treatment. Thus far, most of the studies conducted in citrus have examined the antifungal activities of biocontrol agents after their postharvest application. Considering that pathogen infection may occur in the field before harvest and one of the major modes of action of the biocontrol agents is the competition for nutrients and space, future experiments should be conducted investigating the preharvest application of biocontrol agents in the control of both blue and green molds. Moreover, experiments aiming at identifying and isolating biocontrol agent strains naturally found on the surfaces of citrus species, as well as at determining the environmental conditions promoting their growth, are also encouraged. The combination of non-chemical elicitors or plant growth regulators with the biocontrol agents should be investigated, since it has been previously shown that the efficiency of biocontrol agents can be enhanced when they are combined with others environmentally friendly.

Acknowledgements

The authors would like to thank Dr. Anastasia Ktenioudaki for reviewing and providing comments and suggestions on the manuscript.

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Highlights

- Non-chemical treatments for green and blue mold control
- Essential oils can control the germination of blue and green molds
- Irradiations may effectively control the decay caused by *Penicillium* spp.
- Yeasts and bacteria can been used as biocontrol agents against green and blue molds